WALKER (E.L.)

A COMPARATIVE STUDY

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THE SO-CALLED POLYCHROMATOPHILOUS DEGENERATION OF RED BLOOD CORPUSCLES

ERNEST LINWOOD WALKER

(From the Laboratory of Comparative Pathology of the Harvard Medical School)

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THE SO-CALLED POLYCHROMATOPHILOUS DEGENERATION OF RED BLOOD CORPUSCLES.

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It is well known that during certain pathological conditions in which anæmia is a primary or a secondary factor there appear in the circulating blood red corpuscles showing characteristic morphological and microchemical differences from the normal circulating corpuscles. These "anæmic forms" include corpuscles abnormal in size (microcytes and macrocytes) and in shape (poikilocytes), corpuscles deficient in hæmoglobin, corpuscles having abnormal staining affinities (polychromatophilocytes), nucleated corpuscles (normoblasts, microblasts, and megaloblasts), and corpuscles showing two or more of these changes simultaneously. All of these, with the exception of the normoblasts, are generally believed to be corpuscles that have undergone changes of a degenerative character, either direct necrobiotic changes (polychromatophilia, poikilocytosis, etc.), or else changes resulting from degenerative processes in the hæmatopoietic organ (the megaloblast degeneration of the marrow of Ehrlich), whereby there is a return to a so-called fœtal type of hæmocytogenesis. The normoblasts, being generally recognized as embryonic red corpuscles, are looked upon as representing regenerative changes in the blood. Certain investigators have, however, seen reasons for believing that other of these abnormal corpuscles might belong, with the normoblasts, to the regenerative processes following anæmia rather than to the degenerative changes of anæmia. Their

diagnostic value in addition to their purely biological significance makes a knowledge of the origin and nature of these changes of considerable importance. In this paper there will be considered only those corpuscles that show the so-called polychromatophilous staining, one of the most constant of these changes associated with anæmia.

The discoplasm of the living red corpuscle is said to be achromatophilous; that is, it has no affinity for any of the ordinary histological staining reagents. When, however, the corpuscles are killed and fixed they become chromatophilous; but the stroma of the corpuscles normal to the human blood have an affinity only for those dyes, the staining properties of which reside in the acid part of the staining compound, and are, therefore, spoken of as *acidophilous* (oxyphilous). In anæmic blood, however, the discoplasm of certain of the corpuscles shows a selective affinity for dyes, the staining properties of which reside in the base of the compound, and may consequently be called *basophilous*. These are the polychromatophilous corpuscles of Gabritschewsky, who thus designated them because when stained with the tricolor stain of Ehrlich, or when double stained with eosin and hæmatoxylin or methylene blue, they sometimes stain with a mixture of two or more colors, either as a diffuse mixture or irregularly, some parts of the corpuscle taking color differently from others.

Such diffuse acid stains as eosin do stain all red corpuscles to a certain degree, although the basophilous more feebly than the acidophilous variety. If methylene blue or hæmatoxylin, used as a contrast stain, be applied for only an instant the basophilous corpuscles will present a mixed or polychromatophilous staining due to the fact that the basic stain has not had sufficient time to completely substitute itself for the diffuse acid stain. If allowed to stain several minutes it will entirely supplant the eosin. Methylene blue or hæmatoxylin used alone stain only the basophilous corpuscles, the normal corpuscles remaining entirely unaffected.[1] The

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[1] In preparations of dried blood kept several months the stroma of all the red corpuscles may take up alkaline methylene blue faintly and diffusely. This diffuse absorption is, however, distinguishable from the chemical affinity of the basophilous corpuscles in the same preparation. If the preparation be lightly decolorized with absolute alcohol or very dilute acetic acid (0.1 %) the acidophilous corpuscles are decolorized, leaving the basophilous corpuscles still stained.

other definite basic stains, like methyl green, vesuvin, etc., have a weaker affinity for this variety of cytoplasm. Definite basic stains already mentioned may be used directly or progressively, but certain diffuse basic stains like safranin and fuchsin have to be employed indirectly or regressively for the differentiation of the basophilous corpuscles, and in general give less satisfactory results. The tricolor stain (orange G., acid fuchsin, and methyl green) is unsuitable — Ehrlich to the contrary — for studying these chemical changes in the cytoplasm of red blood corpuscles. Methyl green, the only basic stain in the mixture, having but a feeble affinity for the basophilo-plasm, is submerged by the diffuse acid stains. The more intensely basophilous corpuscles are either more feebly stained than the acidophilous corpuscles or else show a polychromatophilous staining.

This selective affinity for basic stains is more commonly uniform throughout the stroma of the corpuscle, the corpuscle staining a homogeneous diffuse color varying in intensity from the faintest perceptible tint to a moderately deep color; but it sometimes appears limited to certain granules or fragments in the acidophilous stroma, the corpuscle appearing punctate with small stained dots. These differences in staining affinity are microchemical reactions that indicate the different chemical composition of the discoplasm of the two groups of corpuscles, the nature of which will be fully considered in another section. Their staining affinities furnish the easiest means of identifying these corpuscles.

Basophilous corpuscles show slight morphological variations from the acidophilous variety. They are usually larger —in severe anæmias often much larger — than the normal corpuscles. The biconcavity of the disc is generally less marked. And in the much enlarged forms the corpuscle usually has a delicate, attenuated appearance, sometimes associated with irregularity of shape (poikilocytosis).

A review of the literature on the subject shows a diversity of opinion concerning the interpretation of these corpuscles. Smith, Gabritschewsky, and Askanazy, basing their judgment upon comparative and experimental evidence, look upon them as normal phases in the life history of the red corpuscle, and specifically as immature stages of the developing corpuscle, stages which in man are normally passed in the blood-forming tissue of the marrow, and which have

escaped prematurely into the circulation during the active regeneration of the blood following the anæmia. On the other hand, Ehrlich, Maragliano, and their followers, basing their opinion chiefly upon pathological evidence, have been led to believe, since basophilous corpuscles are abnormal to the human circulation and only appear associated with some abnormal, usually pathological condition of the individual, that they represent abnormal phases in the life of the red corpuscle, and, from their association with disease, that they are degenerative changes due to the disease. This is the opinion that seems to have become the more widely accepted and is now generally quoted in text-books as the explanation of them, with the occasional qualification that other and opposing views are held by some.

Ehrlich (5) in 1885 was first to describe corpuscles staining with the basic stains hæmatoxylin and methylene blue, which can be thoroughly identified with the corpuscles that have just been described. He calls them anæmic degeneration forms, and looks upon the change as a sort of coagulation-necrosis in the stroma of the corpuscle. In the same year Favre and Celli (8) described them in malarial blood.

Several observers, including Erb (4), Lowit (13), Foa and Mondino (9), Celli and Guarnieri (3), and Howell (12), some of them prior to Ehrlich, have described granules and parts of corpuscles that stain abnormally, or which were brought out by various treatments of the blood, both from well and from diseased animals and from animals upon which venesection had been practised. It is questionable, however, how far any of these are identical with the corpuscles under consideration.

In the history of a case of purpura presented (by Dr. Prentiss) before the Association of American Physicians in 1890 Theobald Smith was first to diagnose as immature certain red corpuscles staining diffusely with basic stains.

In the same year Gabritschewsky (10) described such corpuscles in cases of anæmia and leukæmia, calling them polychromatophilous corpuscles, for reasons just stated. He also looks upon them as immature corpuscles, on the grounds that the immature nucleated corpuscles of reptiles and birds, and the nucleated corpuscles found in the human circulating blood in anæmia and leukæmia, have a cytoplasm that stains with the basic stain methylene blue. That this is not due to degenerative changes he thinks proven by the occurrence of different phases of mitosis in their nuclei.

In 1891 Smith (17) presented before the Association of American Physicians a paper giving the results of observations upon the anæmic blood

of cattle made during his well-known investigations of the Texas cattle fever. Briefly, the important facts of this paper are as follows:

1. The normal circulating blood of the ox contains only acidophilous normocytes.

2. When through the anæmia of this disease, which is caused by an extensive destruction of red corpuscles by a protozoan endoglobular parasite, as is malaria in man, the number of red corpuscles in the circulating blood is reduced from 5.5–6.5 millions per cmm., the normal number in the healthy animal, to about 3 million, there appear in the circulation a certain number of enlarged corpuscles (macrocytes). When the number has fallen slightly below 3 million, corpuscles with granules staining with basic stains appear. If the destruction still continues, at 1.5–2 million diffusely staining basophilous corpuscles, and at 1–2 million corpuscles per cmm., nucleated corpuscles, are to be found in the circulation. During recovery the disappearance of these abnormal forms from the circulation takes place in inverse order.

3. Smith was able to produce these same phenomena by artificially lowering the number of red corpuscles in the blood through venesection on healthy animals.

4. He considers these abnormal forms to be primarily embryonic corpuscles that have been drawn into the circulation prematurely to supply the increased demand created by the destruction of corpuscles by the parasite.

Askanazy (1) confirms Gabritschewsky that the affinity for basic stains is characteristic of the cytoplasm of nucleated red corpuscles found in the circulating blood, and he finds that the greater number of the embryonic nucleated corpuscles of the human bone marrow, as shown from material obtained from the resection of a rib, show the same selective affinity. The corpuscles of the embryonal human lives, he says, show this same peculiarity.

Since his first communication on the subject Ehrlich has made several contributions to the controversy, always maintaining his belief in the degenerative character of these changes. In his latest work on the blood, in collaboration with Lazarus (7), he summarizes his evidence in support of this theory:

"1. The appearance of those erythrocytes that show the highest degree of polychromatophilia. Through the fragmentation of their edges they appear to every eye trained in the estimation of morphological relations as if in the act of dissolution, as the most pronounced degeneration forms.

"2. The fact that one can cause them to appear in considerable numbers in the blood in animal experiments, for example through inanition, therefore in just the condition when it can be least the question of new building of red corpuscles.

"3. The clinical experience that after acute blood losses in man, even inside of the first twenty-four hours, one can find this staining anomaly in numerous corpuscles, while, according to our very extensive investiga-

tions on this point, embracing many hundred cases and conducted with the greatest care, one finds at this time no nucleated red corpuscles.

"4. Frequently nucleated red corpuscles, especially megaloblasts, show the polychromatophilous degeneration. This is a so easily demonstrated fact that even an untrained observer cannot easily overlook it, and, as is well known, it was Ehrlich who first turned his attention to these relations. But important in their interpretation is the circumstance that the type of the normal regeneration, the normoblasts, are usually free from polychromatophilous degeneration, likewise are the nucleated red blood corpuscles of animals. When Askanazy asserts that the nucleated red blood corpuscles of the bone marrow, which he was able to study in some cases of empyema immediately after resection of the rib, all showed polychromatophilia, this may perhaps be connected with the idiosyncrasies of these cases or with the uncertainty of the staining method employed. Eosin-methylene blue staining is to be denoted as very untrustworthy, since overstaining with the blue is likely to happen. (We expressly advise the use of the tri-acid solution or hæmatoxylin-eosin mixture in the study of anæmic degeneration)."

Ehrlich is supported by Maragliano (14, 15), who studied the changes going on in corpuscles of drawn blood under the microscope, where he observed degenerative changes, including a modification of the staining affinities resembling these changes in the corpuscles in anæmic blood, and from analogy looks upon these latter as necrobiotic.

Maragliano considers these changes in anæmic blood to be due to a toxicity of the plasma. Cabot (2) thinks a lessened resistance to the ordinary plasma environment on the part of the red corpuscles would explain them. Troja (19) looks upon this change in the staining affinity of the protoplasm of the red corpuscle as a sort of karyolysis, whereby a diffusion of the chromatine of the nucleus into the cytoplasm has taken place.

Taking the clue from the investigations of Smith, Gabritschewsky, and Askanazy, my own studies have been made on the blood and bone marrow of vertebrates with the purpose of finding out more of the origin and nature of this change and thereby gaining a better insight into its significance in anæmic blood.

In studying the staining reactions of red corpuscles, Ehrlich dry preparations of the blood and of the marrow were made according to the method described by Smith (17), and subsequently by Mannaberg, Heiman, and Zettnow. The edge of a square cover-glass held in clamp forceps is touched to the drop of blood and then drawn over the surface of a second cover-glass at an angle of 30°, leaving a thin layer of blood that dries almost instantly, fixing the corpuscles in good condition. The ease and

rapidity with which the preparations can be made, the ability to vary the thickness of the preparation to a certain extent independent of the amount of blood taken, and the fact that any slight dirt or grease on the cover-glass is not necessarily fatal to the preparation, make this method preferable to the usual method of allowing the blood to flow between the two cover-glasses by capillarity and then drawing them apart.

Cover-glass preparations thus made and dried in the air at ordinary temperature were fixed half an hour in a mixture of absolute alcohol and ether in equal parts by volume. For the differentiation of basophilous cytoplasm, staining with Löffler's solution of alkaline methylene blue, either alone or as a contrast stain to eosin, gives most satisfactory results. A supplementary use was also made of all of the other appropriate histological stains.

In order to compare the proportion of basophilous or other abnormal corpuscles in the blood or marrow of different species and individuals, counts were made in a microscope field of known area with a Zeiss $\frac{1}{12}$ oil immersion lense. Percentages were determined by differential counts, the average being taken of the counts of one hundred fields. When the proportion of normal to abnormal corpuscles was very great, as in the circulating blood of adult animals, the ratio was obtained approximately by actually counting the abnormal forms and calculating the total number of all forms in a single field. This method gives results of approximate accuracy that are sufficient for our purpose.

Normal Blood of Vertebrates.

Although the appearance of basophilous corpuscles in the circulating blood of man consequent to pathological conditions is well known, less seems to be known and still less significance given to what is known concerning their normal presence in the blood of many species of vertebrates.

Ehrlich (5), in 1885, in the same communication in which he first describes basophilous corpuscles in anæmic human blood, also says he has observed them in the blood of kittens.

W. Erb (4), in 1865, describes granules in red corpuscles of animals, both in normal blood and in the blood after repeated venesection, as well as in the blood of man after severe hæmorrhage or when suffering with chronic disease, which were made apparent by treating fresh blood with 1 % acetic acid or with picric acid.

Löwit (13) observed granulations in red corpuscles of the rabbit when fresh blood from certain vascular territories was heated with a modified Picini's fluid.

Howell (12) describes granules in the corpuscles from the blood of

kittens that had been severely bled which stain with methyl green, and which are considered by him as remains of the nucleus.

Smith (17) briefly mentions that he has observed corpuscles staining with methylene blue in the blood of guinea-pigs, rabbits, mice, and pigeons kept in cages.

I find basophilous corpuscles of the diffusely staining variety to be normal elements of the circulating blood of all the lower species of vertebrates. They are invariably present in small numbers in the blood of snakes, frogs, pigeons, house mice, rats, guinea-pigs, rabbits, cats, and dogs. Their number varies in different species, but is fairly constant in healthy adult animals of the same species. In Table I. are grouped the results of counts of the basophilous corpuscles in the blood of the different species, the percentages in each case being the average of one hundred fields. This table does not represent the number of blood examinations made, but only those in which counts were made from animals taken without selection. The condition of the animals was determined either by post-mortem examination, or, in cases when the animal was not killed, by daily observation. Many of them were animals bred and kept in confinement, or were domesticated animals. Some of them, as the snakes, frogs, rats, and some of the mice, were freshly caught. The pigeons were in confinement but a short time before the blood counts were made.

TABLE I.

Species.	Number.	Percentage of basophilous corpuscles in the circulating blood.	Remarks.
Snake	1	2.73	
Frog	1	14.4	
Pigeon	1	1.84	Healthy adult animal.
Pigeon	2	3.09	Healthy adult animal.
Pigeon	3	3.00	Healthy adult animal.
House mouse	1	1.82	Healthy adult animal.

TABLE I.— *Continued.*

SPECIES.	Number.	Percentage of basophilous corpuscles in the circulating blood.	Remarks.
House mouse..	2	0.64	Healthy animal; ¾ grown.
House mouse..	3	0.40	Healthy adult animal.
House mouse..	4	0.62	Healthy adult animal.
House mouse..	5	1.11	Healthy animal; ⅔ grown.
House mouse..	6	1.44	
Rat	1	Numerous	Healthy adult animal.
Guinea-pig	1	0.065	Healthy animal; weight 477 grams.
Guinea-pig	2	0.041	Healthy animal; weight 335 grams.
Guinea-pig	3	0.03	Healthy animal; weight 271 grams.
Guinea-pig	4	0.26	Tuberculous animal.
Guinea-pig	5	0.19	Tuberculous animal.
Guinea-pig	6	0.094	Pregnant animal.
Rabbit	1	0.22	Adult female; weight 1,800 grams.
Rabbit	2	0.54	Adult male; weight 2,025 grams.
Rabbit	3	1.047	Tuberculous animal.
Rabbit	4	0.838	Tuberculous animal.
Cat	1	0.399	Healthy animal; 6 weeks old; 4 nucleated corpuscles in 100 microscopic fields.
Dog	1	0.163	Healthy adult animal.
Dog	2	0.091	Healthy adult bull-dog.
Dog	3	0.104	Healthy adult pug-dog.
Dog	4	0.478	Pregnant greyhound.
Ox	1	0	Basophilous corpuscles never present in the circulating blood of healthy adult animals.
Horse	1	0	
Man	—	0	

The number of basophilous corpuscles is never large in the normal blood of adult animals, amounting to 2–14% of

all red corpuscles in the blood of reptiles and birds, and rarely reaching 1 % in the blood of the mammalia. Basophilous corpuscles have not been found in the normal blood of the sheep, ox, horse, or man.

The normal presence of basophilous corpuscles in the blood of so many species of vertebrates must mean that they are normal stages in the life history of the red corpuscles in these species, and consequently make us hesitate to believe that they represent degenerative changes due to disease when occurring in the blood of other species. And their normal absence from the circulating blood of some species makes it improbable that they are corpuscles showing degenerative changes due to old age and natural death of the individual corpuscles.

Fœtal Blood.

Opportunity was offered for examining the blood of the fœtuses of a guinea-pig and a dog. Post-mortem examination showed both mother and fœtuses to be healthy. Non-nucleated basophilous corpuscles were present in both cases in numbers vastly in excess of those in the blood of the mother. Normoblasts, megaloblasts, and, in the fœtuses of the dog, corpuscles with basophilous granules, were also present in considerable numbers. Table II. gives the percentages of these different basophilous corpuscles in the fœtal blood, as obtained by differential counts, and the percentages in the blood of the mother, for comparison. In both cases the blood of the mother contained basophilous corpuscles above the average for the species, owing to pregnancy; yet, while these corpuscles in the blood of the mother did not average one in a microscopic field, the blood of the fœtuses showed them so numerous and so prominent because of their large size and deep staining that they appeared at first glance to make up half or two-thirds of all the red corpuscles. Comparison of percentages shows that the blood of the fœtuses of the guinea-pig contained ninety times as many basophilous non-nucleated corpuscles as the blood of the mother, and the blood of the fœtuses of the dog over seventy-two times as many as the blood of the mother.

Smith (17, 18) examined the blood of a three months' fœtus of a cow that had died suddenly of Texas fever. The mother had succumbed so early in the disease that basophilous corpuscles had not made their appearance in her blood, and the blood of the fœtus was not infected with parasites, nor appeared to be diseased in any way. Corpuscles staining diffusely with basic stains were numerous.

TABLE II.

Species.	Number.	Mother.	Fœtus.		Remarks.
		Percentage of non-nucleated basophilous corpuscles in the circulating blood.	Percentage of non-nucleated basophilous corpuscles in the circulating blood.	Percentage of nucleated corpuscles in the circulating blood.	
Guinea-pig	6	0.094	8.48	0.109	
Dog	4	0.478	34.75	0.7	Of the basophilous corpuscles in the fœtal blood 4.89 % contained granular basophilous fragments.

Bone Marrow.

Even more significant are the staining reactions of the corpuscles in the red bone marrow of healthy adult animals. The relative number of non-nucleated basophilous corpuscles in the bone marrow varies in different individuals and in different parts of the same individual, and in general depends upon the activity of the hæmocytogenesis, being greatest in young animals and in those parts of the marrow where the hæmatopoietic function is most active. The selection of preparations of the marrow for making differential counts was therefore guided by the activity of hæmocytogenesis as disclosed by the presence and number of nucleated corpuscles. Differential counts were made of the normal acidophilous, of the non-nucleated basophilous, and of the nucleated corpuscles, and the percentage compared with the

circulating blood of the *same individuals.* The results of these counts are given in Table III.

TABLE III.

SPECIES.	Number.	Percentage of basophilous corpuscles in the circulating blood.	Percentage of basophilous corpuscles in the bone marrow.	Percentage of nucleated corpuscles in the bone marrow.
Pigeon	3	3.00	62.05	62.05
House mouse......	4	0.62	36.06	1.28
House mouse	5	1.11	20.21	1.25
House mouse	6	1.28	53.47	10.56
Guinea-pig........	5	0.19	19.34	9.39
Guinea-pig........	6	0.09	26.34	6.21
Rabbit	4	0.84	23.16	24.26
Cat	1	0.40	34.00	23.9
Dog	4	0.48	34.08	15.06
Dog	5	0.09	27.87	7.08
Dog	6	0.10	30.42	15.72
Ox	1	0.00	12.46	8.85
Horse	1	0.00	Present	Present
Man	–	0.00	——	——

Not only are the large percentages of basophilous corpuscles in the marrow striking, but even more so is a comparison of these percentages with those obtained from the circulating blood of the same individuals. Thus in the pigeon the percentage in the marrow is 20 times as great, in the house mouse 18 to 58 times as great, in the guinea-pig 163 times as great, in the rabbit 27 times as great, in the cat 85 times as great, and in the dog 71 to 306 times as great as in the circulating blood of the *respective animals.*

The blood of the ox, horse, and man, it will be remembered, do not normally contain basophilous corpuscles. The preparations from the marrow of the ox of the one animal

examined contained 12.5 per cent. non-nucleated basophilous corpuscles and nearly 9 per cent. nucleated corpuscles. The circulating blood of this animal did not contain them. The only opportunity to study the marrow of the horse was from an old animal that showed extremely little hæmocytogenesis, at least in that part from which my preparations were made. Of the sixteen preparations made fifteen of them showed no embryonic corpuscles. A single preparation showed a slight activity of the hæmatopoietic function, a few nucleated and about an equal number of non-nucleated basophilous corpuscles being present. The circulating blood of the animal was apparently free from them. Material is not at this time available for the study of the human bone marrow. Askanazy (1), however, says that the greater number of the nucleated red corpuscles in the human marrow, as shown by material obtained from a resected rib, have a polychromatophilous cytoplasm. He found the same to be true of the nucleated corpuscles of the embryonal human liver. But he makes no mention of the staining affinities of the non-nucleated corpuscles of the human marrow.

The universal presence of basophilous corpuscles as elements of the embryonic red corpuscle tissue in the healthy vertebrate marrow furnishes unqualified disproof that they are corpuscles showing changes due to pathological conditions.

The Relation of the Affinity for Basic Stains to the Development of Hæmoglobin.

In the hæmatopoietically active bone marrow of mammals there are, beside the marrow cells proper and the leucocytes, four types of cells morphologically distinguishable that will be generally accepted as representing different stages in the development of the red blood corpuscle. There are (a) normal non-nucleated corpuscles like that of the circulating blood; (b) large non-nucleated corpuscles (macrocytes); (c) what may be designated as mature nucleated corpuscles, *i.e.*, corpuscles morphologically like the non-nucleated red corpuscles, except for a medium-sized spherical nucleus in the

otherwise homogeneous cytoplasm; and (d) young nucleated corpuscles in different stages of development.

The younger erythroblasts are comparatively large cells, with a somewhat granular cytoplasm, having a large nucleus with a loose reticulated structure occupying the larger portion of the cell. Transitional forms, showing every intermediate stage between these cells and those designated as the mature nucleated corpuscles, are present. In a single microscopic preparation, sometimes in a single microscopic field, there may be seen nucleated corpuscles showing every possible gradation, so that the relation of the different stages is unquestionable. The progressive morphological changes accompanying the development of these embryonic corpuscles are a decrease in size of the whole cell, an increase in the homeogeneity of its cytoplasm, a decrease in size of the nucleus both relatively and absolutely, the nucleus coming to occupy less of the cell in the mature nucleated corpuscle, and an increase in the density of the nuclear substance. The mature nucleated corpuscle is acknowledged to give rise to the non-nucleated red corpuscle through the loss of its nucleus, either by extrusion or by absorption. One often sees mature nucleated corpuscles with partially or wholly extruded nuclei, whose cytoplasm is morphologically indistinguishable from the non-nucleated red corpuscle.

Accompanying these morphological changes attending the development of the red corpuscle are parallel chemical changes. The nucleus, with its increasing density of structure, stains more and more uniformly and intensely with the chromatin stains. The cytoplasm of the younger nucleated corpuscles is an unmodified protoplasm containing no hæmoglobin. With the development of this embryonic cell there goes on a gradual transformation of the embryonic cytoplasm into the hæmoglobiniferous discoplasm of the mature red corpuscle. This is manifested by the appearance and gradual intensification of hæmoglobin color in the cytoplasm of the more and more mature corpuscles. But while morphological maturity is practically attained with the loss of the nucleus, complete hæmoglobin development is not usually reached

until some time afterwards. An examination of an unstained preparation of the hæmatopoietically active mammalian marrow will show the greater number of the large non-nucleated corpuscles to be deficient in hæmoglobin color. These macrocytes show all degrees of hæmoglobin development, from the faintest perceptible tint to the deep color of the normal circulating corpuscle. In general, it may be said that these non-nucleated macrocytes take up the degree of hæmoglobin color attained by the mature nucleated corpuscles, and extend it through all distinguishable gradations to the full color of the mature corpuscle. These macrocytes would thus seem to be corpuscles slightly immature, some of them not having attained complete hæmoglobin development, and others, although chemically mature, are still morphologically immature. Occasionally corpuscles of normal size show incomplete hæmoglobin development. And rarely hæmoglobin development is completed before the loss of the nucleus.

This tendency for the hæmoglobin development of the corpuscle to lag behind the morphological development seems to be connected with the activity of the hæmatopoietic function. Hæmocytogenesis is more extensive and active in fœtal than in extra-uterine life, in young than in old animals, in animals that have suffered an abnormal loss of red corpuscles than in normal animals, and, apparently, in the lower than in the higher species of vertebrates. In all of these cases the chemical development of the corpuscle shows a corresponding greater tendency to fall behind the morphological development; as if the time necessary for the complete transformation of the embryonic cytoplasm into the hæmoglobiniferous stroma was nearly constant, while any acceleration of the hæmatopoietic function hastened the morphological development of the corpuscle.

If, with the microscopic field of an unstained marrow preparation in focus, a drop of Löffler's alkaline methylene blue solution be allowed to flow between the cover-glass and the slide the cytoplasm of all the corpuscles deficient in hæmoglobin will instantly take on the diffuse bluish color

characteristic of basophilous corpuscles stained with this solution, while the mature, deeply hæmoglobin colored corpuscles remain unaffected, standing out in the blue background in strong contrast as bright yellow discs. If careful notice be taken previous to the staining of the depth of hæmoglobin color of the different corpuscles in the microscopic field it will be seen that the intensity with which the cytoplasm of the different corpuscles stain with the basic stain varies inversely as their hæmoglobin development. The maximum stain is seen in the most immature nucleated corpuscles with unmodified cytoplasm. It decreases with the increasing hæmoglobin color, and, since complete transmutation of the cytoplasm of the erythroblast into the hæmoglobiniferous stroma of the circulating corpuscle is not ordinarily completed until some time after the loss of the nucleus, it extends over to these non-nucleated corpuscles. Just as every possible gradation in hæmoglobin color, from the faintest tint to the deep color of the mature corpuscle, is to be seen among these non-nucleated corpuscles of the marrow, so do they display *in inverse order* every possible gradation in intensity of staining with basic stains. It is consequently possible, in an unstained microscopic preparation of the marrow, to recognize from the depth of hæmoglobin color not only those corpuscles that are basophilous, but also the degree of their affinity for basic stains; and, conversely, in a stained preparation, from their affinity for basic stains are we able to tell the degree of hæmoglobin development the corpuscle has attained.

There may, then, be distinguished four type phases in the development of the mammalian red blood corpuscle:

a. Young nucleated corpuscles. *b*. Mature nucleated corpuscles.[1]	Deficient in hæmoglobin; basophilous.
c. Non-nucleated macrocytes.	Basophilous or acidophilous.
d. Non-nucleated normocytes.	Hæmoglobin fully developed; acidophilous.

[1] Occasionally acidophilous, hæmoglobin development being completed before the loss of the nucleus.

The transitional forms connecting these different stages are so numerous and the gradations so continuous that the relation of the different stages to each other and to the degree of development is unmistakable.

This is even more clearly brought out by a study of the marrow of the lower vertebrates, as of the pigeon, where the red corpuscle is nucleated throughout its life. The embryonic cells of the marrow in the pigeon, from which the red corpuscles are developed, are large cells that are spherical, or polyhedral from mutual contact, having a somewhat granular cytoplasm and a large spherical nucleus with a reticulated structure occupying the greater part of the cell. The cytoplasm of these cells is entirely free from hæmoglobin, and is strongly basophilous. With development the cell decreases in size and becomes oval, almost lenticular, and its cytoplasm shows an increasing homogeneity. The nucleus becomes smaller, both relatively and absolutely, takes on an oval and finally narrow lenticular outline, and shows an increasing density in structure and intensity in staining. With these morphological changes go the parallel chemical changes in the cytoplasm: a gradual transformation of the basophilous protoplasm of the embryonic cell into the acidophilous, hæmoglobiniferous stroma of the mature corpuscle, manifested by an increasing depth of hæmoglobin color and a decreasing intensity in its affinity for basic stains.

The shape of the nucleus especially enables us to recognize the finer differences in the degree of development in the nearly mature avian corpuscle, the spherical nucleus of the embryonic corpuscle becoming progressively more and more elongated and pointed as the corpuscle approaches maturity, becoming narrow lenticular in the mature corpuscle. Any corpuscle with a nucleus showing any departure from the narrow lenticular outline towards the oval, be it ever so slight, will be accompanied by a corresponding degree of chemical difference in its cytoplasm, a lessened degree of hæmoglobin color, and an increased affinity for basic stains. Turning to the circulating blood of the pigeon, we have a morphological as well as a chemical (hæmoglobin) index to

the maturity of the corpuscle. Judged by this morphological criterion, as well as by the hæmoglobin development, the basophilous corpuscles in the circulating blood of the pigeon are immature corpuscles whose cytoplasm is incompletely transformed into acidophilous hæmoglobin. This relation of basophilous staining to the morphological development of the corpuscle in reptiles and birds is well brought out in the colored plates illustrating Gabritschewsky's first article on this subject in 1891 (10).

Both Ehrlich (5) and Maragliano (14, 15) call attention to the deficiency in hæmoglobin of the basophilous corpuscles in anæmic human blood. But these authors have looked upon the phenomenon as a degenerative change in the discoplasm of the mature corpuscle whereby the acidophilous hæmoglobin is converted into, or replaced by, a basophilous substance, instead of a process in which embryonic basophilous cytoplasm is being transformed into acidophilous hæmoglobin in the development of the corpuscle.

The Conditions of the Appearance of Embryonic Red Corpuscles in the Circulating Blood.

In all but a few of the higher mammalia immature red corpuscles regularly enter the circulation during the physiological regeneration of the blood. Basophilous non-nucleated corpuscles are constantly present in the circulating blood of all of the lower species of vertebrates examined. Newman (16) states that nucleated red corpuscles are always present in small numbers in the blood of the pig. Howell (12) says that the same is true in the blood of the opossum. Why red corpuscles should enter the circulation before they are capable of performing their proper function, since they are yet at most only incompletely supplied with hæmoglobin, and thus load the blood to a certain extent with more or less useless corpuscles, is not apparent. Whether all new red corpuscles undergo their final stages of hæmoglobin development in the circulation of these species, or whether only a part accidentally escape with the other mature corpuscles into it, and, if accidentally present, whether they complete their develop-

ment after entering the circulation, or whether they never attain it, but live out their lives as useless corpuscles, are questions that cannot be answered with certainty. It would seem possible, however, that in the lower vertebrates the red corpuscle completed its final stages of hæmoglobin development, at least in some of the corpuscles, after entering the circulating blood, instead of in the marrow, as is the case with the higher mammalia. Indeed, these are indications that as we descend the scale of development there is a tendency for more and more of the final stages of the development of the red corpuscle —that is normally completed in the hæmatopoietic organ in the higher mammalia — to be carried on after the corpuscle has entered the circulation. Basophilous corpuscles with incomplete hæmoglobin development, absent in the higher mammals, are more and more prevalent as we descend the scale of classification. Nucleated corpuscles are said to constantly enter the circulating blood of some of the lower mammalia. And among the lower vertebrates, the aves and reptilia, the corpuscles are nucleated throughout their life. Embryological evidence points in the same direction. The red corpuscles of the very young mammalian fœtus are all nucleated. In the nearly developed fœtus nucleated corpuscles are still present, but comparatively few in number. But the non-nucleated corpuscles show pronounced embryonal characters: marked enlargement, deficiency in hæmoglobin, and strong basophilous staining. In extra-uterine life the nucleated corpuscles disappear and the non-nucleated corpuscles show less and less pronounced embryonal characters, and may lose them entirely.

In certain of the higher mammalia, including man, immature red corpuscles are usually restricted to the bone marrow, but under certain conditions they do appear in the circulating blood. Observation and experiment have determined these conditions to be any disturbance of the ratio between supply and demand of new red corpuscles.

In the normal adult physiological regeneration of the blood hæmocytogenesis and hæmocytolysis balance; new red corpuscles are matured and turned into the blood current as

fast as is required to make good the loss from the natural death of old corpuscles. During fœtal life there is an excessive hæmocytogenesis accompanying fœtal development; red corpuscles are formed faster than they are matured. Immature red corpuscles are consequently *forced* into the circulation and are always present in fœtal blood.

Ehrlich, Müller, Rindfleisch, Engel, etc., while not agreeing as to the details of the changes in the marrow, all look upon the pernicious anæmias as due to defective and deficient hæmocytogenesis. Some pathologists, however, conceive these anæmias to be due rather to excessive hæmocytolysis. The so-called secondary anæmias may result from blood losses through hæmorrhage, destruction of red corpuscles by parasites, or through other excessive hæmocytolysis, or they may be due to defective hæmocytogenesis, resulting from deficient nutrition, etc. But whether these anæmias be primary or secondary, due to deficient hæmocytogenesis, to excessive hæmocytolysis, or to direct blood losses, the demand for new red corpuscles exceeds the supply, and incompletely developed corpuscles are *drawn* into the circulation in attempt to satisfy this demand.

This is the explanation advanced by Smith (17) in 1890, considering their presence in the blood of cattle consequent to the pernicious anæmia of Texas cattle fever: "We shall not be far from the truth, I think, in assuming that we have here unfinished or embryonic corpuscles sent into the circulation before their time to make good the losses going on. We are led from the enlarged but otherwise normal corpuscles through those containing granules and staining diffusely to the hæmatoblast. Each stage is more embryonic than the preceding, and called into active service by more and more pressing need. We are, however, justified in asking whether these stages are those which the corpuscles ordinarily pass through, or whether pathological conditions are superadded. It is highly probable that there is both an embryological and a pathological factor involved." This last statement is true with reference to the aggregation of basophiloplasm and to the overgrowth of the corpuscles in extreme anæmia. With

these exceptions we now know these "anæmia corpuscles" to be normal stages of development of the red corpuscle that are always present in the hæmatopoietically active marrow.

It is in this regard that Ehrlich (6) arrives at mistaken conclusions concerning the appearance of basophilous corpuscles in the blood of starved dogs, and in cases of post-hæmorrhagic anæmia at a period so early that, as he thinks, new corpuscles could not yet have had time to be formed. It is, however, not a question of the formation of new corpuscles, but of drawing into the circulation immature corpuscles that are already present in certain parts of the marrow as successive stages in the development of new red corpuscles required in the physiological regeneration of the blood. It is also probable that under the excitation of an increased demand the hæmatopoietic organ is stimulated to an increased activity in the production of new corpuscles.

The same conditions that cause red corpuscles to escape prematurely from the marrow into the circulating blood of species that are normally free from them also increases their number in the blood of those species that normally contain them in small numbers. The effect of anæmias secondary to tuberculosis and to pregnancy on the number of basophilous corpuscles in the blood of guinea-pigs, rabbits, and dogs is shown in Table I., where a comparison may be made with the number in the blood of normal animals of these species.

The significance of the basophilous substance in punctate or granular fragments in an oxyphilous stroma in certain corpuscles of anæmic blood is not understood. In all of the embryonic forms in the healthy marrow, in fœtuses, and in the circulating blood of the lower species of vertebrates the transmutation of the basophilous cytoplasm into the oxyphilous hæmoglobin takes place gradually and uniformly, the only exception to this being in the blood of the fœtuses of dog number 4, in which corpuscles with basophilous granules are numerous. On the other hand, in anæmias, both of man and of other animals, these punctate forms are characteristic. In the blood of cattle in the progressive oligocythæmia of Texas fever, and from venesection, they are always the first form of

basophilous corpuscle to appear, followed by the diffusely staining variety. In all of these cases — with the exception of the dog fœtuses just mentioned — the presence of basophilous corpuscles in the blood is abnormal; and since this punctate variety is not found among the developing corpuscles in the marrow or in the fœtus, at least in healthy animals, they are under the suspicion of being the products of abnormal conditions. Smith (17) suggests that these granules may be derived from the diffusely staining cytoplasm through a condensation or aggregation of the basophilous substance in the circulation.[1]

The practical value of the foregoing is that there is furnished a simple, quick, and at the same time fairly accurate means of microscopic diagnosis of anæmia. By the application of an extremely simple staining method to dried blood films, and without the use of the more complicated apparatus and technique necessary in making blood-corpuscle counts in fresh blood, the presence or absence of anæmia may be quickly determined. Exception might be made to possible cases in which a decrease of red corpuscles below the normal is not followed by an increased demand upon the hæmatopoietic organ, the organism living, so to speak, upon a lower plane of vital activity. I am not aware, however, that such conditions have been proven to exist. The degree of the anæmia or of the blood loss in hæmorrhage is roughly indicated by the number and phase of development of the embryonic corpuscles in the circulation, their number and

[1] After this had gone to the printer two papers by Plehn and Litten in recent numbers of the Deutch med. Wochenschrift have come to my notice.

Plehn (20) believes the granules and fragments in corpuscles in the blood in tropical anæmias that stain with hæmatoxylin (in Ehrlich's acid hæmatoxylin-alum-eosin) and with methylene blue to be spore or embryo stages of the malaria parasite. Litten (21) hesitates between two, to him possible, origins of the basophilous granules in red blood corpuscles in severe anæmias: (1) a karyolytic process ("kernzerfall"), and (2) a degenerative process in the hæmoglobin-holding protoplasm. He finally favors the first process. Ehrlich also considers them to be the product of disintegration of the nucleus of the megaloblast.

Smith's studies of the blood in the Texas fever (17, 18) fully explain the relation of these granules to the anæmia and to the hæmocytozoan parasite, and refute Plehn's suppositions. That basophilous granules are not the product of disintegration of the nucleus is proven by their presence, not only in the non-nucleated corpuscles, but also in normoblasts with intact nuclei and in the immature nucleated corpuscles. Moreover, their microchemical reactions show them to be basophilous but not chromatin, and in this identical with the cytoplasm of the embryonic red corpuscle.

immaturity increasing with the severity of the anæmia. In mild secondary anæmias they are few in number and present simply slight enlargement and incomplete hæmoglobin development associated with basophilous staining. In paludal and especially in the primary pernicious anæmias they may become very numerous, include nucleated forms, and present abnormal phases of development. By a more careful comparison of the results of red corpuscle counts with the number and phase of development of the embryonic corpuscles in anæmic human blood, as was done by Smith in the blood of the ox in Texas fever, one might be enabled to estimate the degree of oligocythæmia within narrower limits.

When considered with the clinical and microscopical evidence, these embryonic red corpuscles may, if judiciously employed, furnish important aid in differential diagnosis. They are constantly made use of in this laboratory as a supplementary guide in the microscopical diagnosis of malaria. This disease, being associated with a more or less extensive destruction of red corpuscles caused by the specific hæmocytozoan parasite, presents a more pronounced oligocythæmia than is likely to be associated with other febrile diseases, or diseases that have febrile symptoms, that might be mistaken clinically for malaria. Consequently, the presence of immature corpuscles in the blood of suspected cases, especially if they are numerous, would lead to the suspicion of malarial infection even when the blood be taken for examination at a time when the parasites were relatively few in the peripheral circulation. In such cases a more careful search is demanded, and is sometimes rewarded by the discovery of one or more parasites that would otherwise have been overlooked. Corpuscles containing punctate or granular fragments of embryonic basophiloplasm seem to be very characteristic of the severer grades of paludal anæmias. On the other hand, the absence of embryonic red corpuscles from the blood would help confirm a negative diagnosis.

For diagnostic purposes the following simple technique is recommended: Dried blood films are to be fixed 15 to 30 minutes in a mixture of equal parts of absolute alcohol and

ether, stained 5 to 10 minutes with Löffler's alkaline methylene blue solution, and examined in water. The nuclei of the white and the red corpuscles, the basophilous corpuscles, and malaria parasites, if present, are stained blue. The normal red corpuscles are unstained.

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